'Click for PET'

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Chapter 4
Tandem Enantioselective Biocatalytic Epoxide Ring Opening and [3+2] Azide Alkyne Cycloaddition

Halohydrin dehalogenase HheC can perform enantioselective azidolysis of aromatic epoxides to 1,2-azidoalcohols which are subsequently ligated to alkynes producing chiral β-hydroxytriazoles in a one-pot procedure with excellent enantiomeric excess. This method uses inexpensive and readily available racemic epoxides and gives good results with both the traditional copper(I) catalyzed 'click' reaction as well as with the copper-free variant. A tracer based on the β-azidoalcohol motif was designed for targeting cerebral β-adrenergic receptors.

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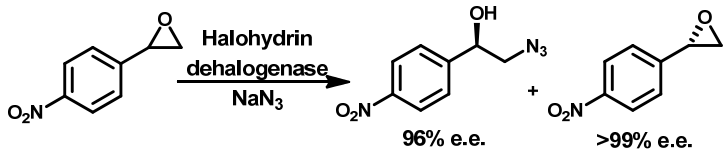
Radiochemical reactions and *in vitro* studies were performed by Leila Mirfeizi

4.1 Introduction

Recently, efforts have been pursued to involve the copper catalyzed azide alkyne cycloaddition (CuAAC) in one-pot multicomponent reactions,[1] and interest in broadening the scope of these reactions has emerged.[2] The bioorthogonality of the azide alkyne cycloaddition makes it uniquely suited to one-pot procedures and as such should be exploited in tandem processes.

An attractive possibility to execute a tandem reaction is the combination of azide induced ring opening of epoxides with the CuAAC. Starting from enantiomerically pure epoxides, it has been shown that azidolysis followed by the 'click' reaction can occur in one-pot with PEG-400 as a solvent, with retention of the enantiomeric excess of the starting material.[3] Another approach demonstrated the possibility of enzymatically reducing α-azidoacetophenone derivatives in an enantioselective manner to their azidoalcohol counterparts. As both the acetophenones and the resulting alcohols contain the azide functionality, the alcohol must first be isolated prior to attachment to an alkyne via copper catalysis.[4]

Halohydrin dehalogenases are a class of enzymes that catalyze the expulsion of a halide and a hydrogen to form an epoxide ring through intramolecular ring closure. It has been shown that they are also able to catalyze the reverse reaction, *i.e.* the ring opening of an epoxide with a variety of halides including chloride, bromide and iodide in a β-regioselective fashion.[5] Further still, as a class of enzymes, the halohydrin dehalogenases prove to be quite promiscuous, catalyzing the ring opening with many other anionic nucleophiles including cyanide, cyanate, thiocyanate, formate, nitrite and azide.[6] It has been reported that biocatalytic azidolysis of aromatic epoxides using HheC, the halohydrin dehalogenase from *Agrobacterium radiobacter* can be performed with excellent regio- and enantioselectivity.[7] For instance, the azidolysis of substituted styrene oxides to their corresponding chiral 1,2-azidoalcohols can be catalyzed by HheC in a highly enantioselective (E>200) and *β*-regioselective manner (Scheme 1).

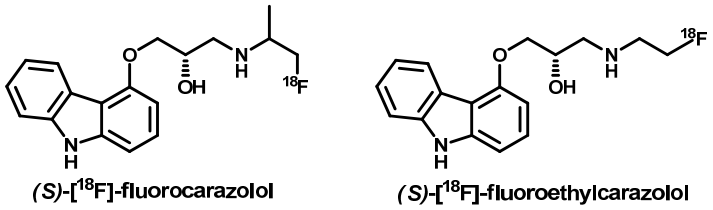


Scheme 1 Biocatalytic azidolysis of nitrostyrene oxide

HheC has been cloned, and brought to overexpression, lending it potential for industrial scale, therefore rendering biocatalysis by HheC a versatile method for synthetic organic chemists.[8] The enzyme is sensitive towards oxidation and as such, β-mercaptoethanol is often a necessary additive to stabilize the enzyme. However, β-mercaptoethanol is a potential nucleophile for the ring opening of epoxides. A variant of HheC with cysteine 153 mutated into serine can be constructed, increasing the enzyme's stability towards oxidation and eliminating the need for the addition of β-mercaptoethanol.[9]

It was envisioned that the exquisite selectivity of the enzyme (Scheme 1) could be combined with the copper catalyzed [3+2] cycloaddition of azides and alkynes to produce optically pure triazoles. These products are interesting, not only due to the presence of the 1,2,3-triazole moiety which has proven to be a promising pharmacophore[10] but also due to their possible role as *β*-adrenergic receptor blocker analogues illustrating their potential as imaging agents for positron emission tomography.[11]

Cerebral *β*-adrenergic receptor (β-AR) concentrations are linked to a wide variety of psychiatric illnesses, including depression,[12] schizophrenia[13] and Alzheimer's disease,[14] amongst others. Quantifiable visualization of receptor densities would provide a better understanding of the progress and mechanism of any one of the aforementioned diseases. Radiotracer-based imaging in the brain presents particular challenges, especially regarding penetration of the blood-brain barrier (BBB). A tracer cannot be too hydrophilic, or it will not cross the BBB. However, increased lipophilicity also increases the non-specific binding of the compound in question. Ideally, the log P (logarithm of the partition coefficient) value of a compound should fall between 2 and 3 for optimal BBB penetration.[15] Among the multitude of potential tracers, the carazolol class of radioligands has shown promising results. Two compounds in particular, (*S*)-[^{18}F]-fluorocarazolol and (*S*)-[^{18}F]-fluoroethylcarazolol (Scheme 2), showed high specific binding to β-ARs in rodent brains and the former showed specific binding in human brains.[16] However, it was later discovered that both compounds are highly mutagenic, and they were no longer pursued.[17] To date, no ligand for imaging β-ARs in the human brain is available.



Scheme 2 Carazolol tracers for cerebral β-AR imaging

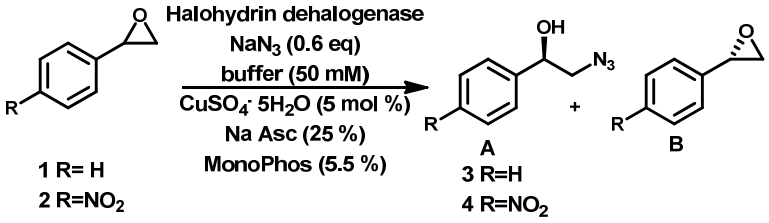
4.2 Goal

The aim of this research was to exploit the robust regio- and enantioselectivity of the HheC enzyme to form optically pure vicinal azidoalcohols and to develop a system which allows for a subsequent one-pot CuAAC to produce the corresponding optically pure triazoles. The challenge was to produce conditions which allowed for a CuAAC to occur in tandem with a highly selective biotransformation performed by an enzyme. A further aim of the project was to develop potential β-AR ligands for positron emission tomography based upon the carazolol motif through the incorporation of an azidoalcohol functionality. This would provide a handle for straightforward introduction of a radioisotope, such as [^{18}F], through a copper catalyzed azide alkyne cycloaddition to allow for a wide variety of structurally similar tracers to be produced and tested.

4.3 Optimization of Reaction Conditions

The investigation was initiated by exploration of the catalytic properties and selectivity of the enzyme in the presence of 'click' additives, namely, $CuSO_4 \cdot 5H_2O$, the reducing agent sodium ascorbate and MonoPhos used to enhance the rate of the CuAAC as previously described (see Chapter 3).[18] In anticipation of the one-pot reaction, the buffer used to store the enzyme, 10 mM Tris-HCl (pH 7.5, 1.0 mM EDTA, 10% glycerol), had to be changed due to the presence of ethylenediaminetetraacetic acid (EDTA) which has the ability to chelate copper, thereby inhibiting catalysis.[19] Potassium phosphate buffer was chosen as a substitute (pH 7.5, 50 mM). Styrene oxide **1** was used as the initial substrate.

Table 1 Enzymatic activity in the presence of 'click' additives



	R	Concentration[a]	Solvent[b]	Additives[c]	Conversion[d]	ee A (%)	ee B (%)	E
1	H	2.0	Buffer	No	47%	>99	89	>200
2	H	2.0	Buffer	Yes	26%	>99	35	>200
3	NO_2	2.0	Buffer	No	46%	>99	83	>200
4	NO_2	2.0	Buffer	Yes	50%[e]	97	98	>200
5	NO_2	4.0	Buffer	No	17%	>99	20	>200
6	NO_2	25.0	Water	Yes	<1%	n.d.	n.d.	n.d.
7	NO_2	25.0	Water	No	<1%	n.d.	n.d.	n.d.
8	NO_2	50.0	Buffer	No	<1%	n.d.	n.d.	n.d.

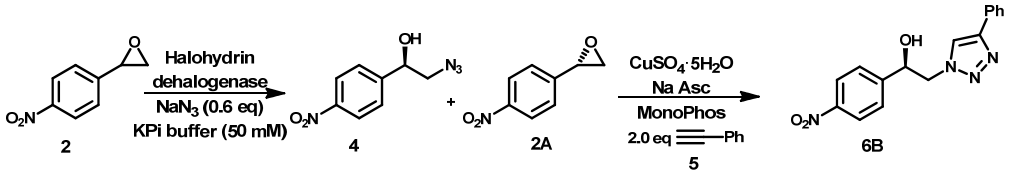
[a]Epoxide concentration (mM). [b]Reactions were conducted either in 50.0 mM potassium phosphate buffer (pH= 7.5) or in distilled water. [c]Includes $CuSO_4 \cdot 5H_2O$, sodium ascorbate and MonoPhos. [d]Conversion at 16 h. [e]Conversion after 24 h.

In Table 1, the catalytic behaviour of HheC in various conditions has been summarized. Fortunately, changing the buffer had no apparent impact on the resulting enantiomeric excess of azidoalcohol **3** (99% ee) (entry 1). The same reaction was performed in the presence of $CuSO_4 \cdot 5H_2O$ (5 mol%), sodium ascorbate (25 mol%) and MonoPhos (5.5 mol %) (entry 2).[20] The presence of these additives proved to have no effect on the selectivity of the enzyme (E>200), but we found that the rate decreased considerably in their presence (26% conversion). The conversion of 2-(4-nitrophenyl)oxirane **2** to its corresponding azidoalcohol **4** in potassium phosphate buffer was also investigated (entry 3). Nearly full conversion was achieved overnight with excellent selectivity (E>200) yielding the desired product **4** with >99% ee. The same reaction in the presence of the additives (entry 4) showed full conversion to **4** after 24 h with 97% ee and 98% ee for the azide and the epoxide, respectively. A higher substrate concentration (4.0 mM) proved to have a detrimental effect upon the conversion while retaining the perfect enantioselectivity of the transformation (entry 5).

Previous work has shown us that the 'click' step of the cascade proceeds faster in distilled water than in potassium phosphate buffer.[21] However, when the enzymatic conversion to azidoalcohol **4** was attempted in water, no product formation was detected (entry 6). In the absence of 'click' additives, the same result was observed (entry 7). The poor result is thus attributed to the inability of the enzyme to perform under these buffer-free conditions. High substrate concentration (50 mM) also proved too challenging for the enzyme, and no trace of **4** was detected (Table 1, entry 8). There was also some concern that the presence of a copper salt could facilitate diol formation. Thus the epoxide was allowed to stir alongside the additives in potassium phosphate buffer in the absence of enzyme for 48 h, at which point no evidence of diol formation was seen, and the starting material could be recovered quantitatively.

Once the ability of the reaction to proceed in potassium phosphate buffer in the presence of the necessary additives with essentially unaltered selectivity had been established, it was possible to attempt the one-pot ring opening and subsequent 'click' reaction with model alkyne phenylacetylene **5** (Table 2). In the first attempt, with 5 mol % of catalyst, after 24 h, the triazole product **6B** could be detected with 99% ee, and the remaining epoxide with 75% ee (entry 1). Thus, the first step of the cascade maintains its high level of selectivity, and the 'click' reaction proceeds at such a rate that with 5 mol % of copper, no azidoalcohol remained in the reaction mixture.

Table 2 Optimization of one-pot enzymatic and 'click' reactions



	Conc. (mM)	Cu (mol %)	Time (h)	Conv.	ee 2A (%)	ee 6B (%)	E
1	2.0	5	24	43%	75	>99	>200
2	4.0	5	24	34%	51	97	109
3	4.0	5	67	39%	62	97	124
4	4.0	1	24	n.d.[a]	85	99	>200
5	50.0	5	67	24%	23	42	2.8

[a]Azidoalcohol remaining.

However, there was only 43% conversion after 24 h. We determined that the enzymatic conversion of 2-(4-nitrophenyl)oxirane **2** to azidoalcohol **4** still proceeded to 50% in 24 h (Table 1, entry 4) in the presence of the catalytic additives ($CuSO_4 \cdot H_2O$, sodium ascorbate

and MonoPhos). Thus the conversion appears to be affected either because of the presence of phenylacetylene, or because of one of the intermediate species in the catalytic cycle of the CuAAC (Chapter 1, Scheme 2).

The same experiment at a higher concentration showed a slight drop in the ee of **4** (97 %) and lower conversion (entry 2). Repetition of the experiment with a longer reaction time gave a slight increase in conversion (entry 3). Reducing the amount of copper catalyst to 1 mol % also gave excellent results, 99% ee for **5B** and 85% ee for the remaining epoxide (entry 4). Comparing entries 2 and 4 it can be ascertained that the concentration of the catalytic additives affects the selectivity of the enzyme. With 1 mol % of copper, the copper complex concentration is lowered (from 0.20 mM to 0.04 mM) and the enantiomeric excess rises. However, detection of azidoalcohol **4** in the reaction mixture indicates that with 1 mol % of catalyst the cycloaddition slows to the extent that it becomes the rate limiting step in the cascade.

Of particular interest is a reaction performed at 50 mM concentration (entry 5). As aforementioned, no trace of **4** had been detected when the enzymatic transformation was attempted at such high substrate concentration (Table 1, entry 8). However, in the presence of 'click' additives and phenylacetylene, after 24 h, 24% conversion to triazole **6B** was detected. Although the ee was lower (42%) in the product than usual, it is significant that the occurrence of the second reaction appears to promote formation of azidoalcohol in the first reaction.[22] Overall, with optimized one-pot conditions, it is possible to achieve 43% conversion to the β-hydroxytriazole with >99% ee.

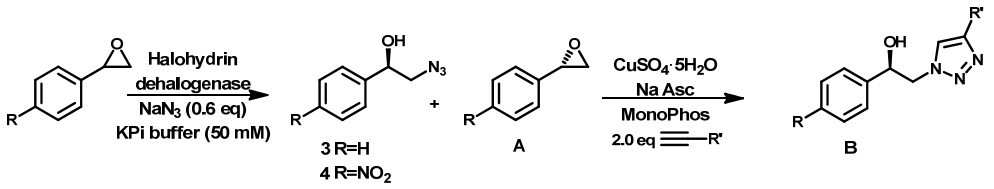
4.4 Substrate Scope

Curious as to the effect of the structure of the epoxide and the alkyne upon the outcome of the reaction, a selection of substrates was made for further investigation (Table 3). In the instance of styrene oxide **1**, the results were very satisfying. At 4.0 mM substrate concentration, the corresponding triazole was detected with > 99% ee, and conversion from epoxide to product was 44%, with 78% ee for the epoxide (entry 1). This is an interesting result when compared with the result from Table 1, entry 2 which showed a lower conversion and enantiomeric excess in the azidoalcohol. This is another instance, as before, where the addition of the second step of the cascade appears to promote conversion in the first reaction (the ring opening of the epoxide). Reducing the catalyst loading from 5 to 3 % again proved insufficient, as azidoalcohol **3** remained in the reaction mixture (entry 2).

To investigate the effect of a different alkyne on the reaction cascade, propiolic acid was also tested (entry 4). Interestingly, the triazole product was racemic. We hypothesized that the presence of the acid functionality could impact the functionality of the enzyme. Thus the corresponding ester, methyl propiolate, was tested as well (entry 5). Indeed, the resulting triazole showed a dramatic improvement in ee to 80% but the conversion of

epoxide remained low after 24 h. We concluded from these observations that not only is the epoxide important as the substrate undergoing enzymatic conversion, but the choice of accompanying acetylene is equally relevant for both rate and selectivity.

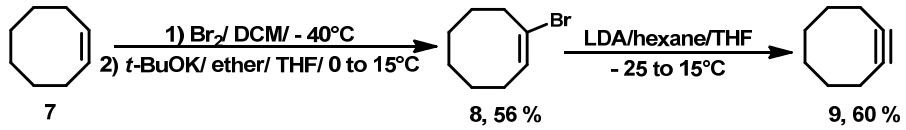
Table 3 Substrate scope



	R[a]	R'	Cu (mol %)	Conv.[b]	ee A (%)	ee B (%)	E
1	H	Ph	5	44%	78	99	>200
2	H	Ph	3	n.d.[c]	78	98	>200
3	NO_2	Ph	5	34	51	97	109
4	NO_2	COOH	5	n.d.	n.d.	2	n.d.
5	NO_2	COOMe	5	20[d]	20	80	10

[a]4.0 mM substrate concentration. [b]After 24 h. [c]Azidoalcohol remaining in the reaction mixture. [d]Enzyme added in two portions (at 0 h and 12 h).

Given the success of the one-pot cascade with the traditional copper catalyzed [3+2] azide alkyne cycloaddition, the more biologically relevant copper-free 'click' reaction was attempted. Cyclooctyne was chosen as a model substrate, and prepared from readily available *cis*-cyclooctene (Scheme 3).[23]

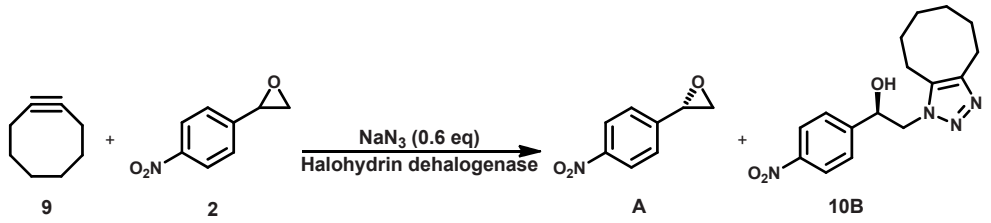


Scheme 3 Synthesis of cyclooctyne

Dibromination was achieved using bromine in DCM at -40°C, followed immediately by HBr elimination by potassium *tert*-butoxide in a mixture of ether and THF. The mono-brominated product **8** was purified by distillation, after which it was treated with lithium diisopropylamide (LDA) at low temperature. Cyclooctyne was isolated by a second distillation step. The enzymatic reaction in the presence of cyclooctyne, and in the absence of the regular additives, was allowed to proceed for 24 h.[24] Analysis by HPLC revealed the

triazole with 96% ee, and the epoxide with 24% ee, indicating 20% conversion (Table 4, entry 1).[25] The reaction was repeated over 48 h, adding the enzyme in two portions (half at the start of the reaction, and the other half after 24 h) to ensure constant enzymatic activity. This resulted in a significant increase in the ee of the epoxide to 47% along with an improvement in conversion.

Table 4 Copper free cycloaddition of cyclooctyne to *in situ* generated azidoalcohol



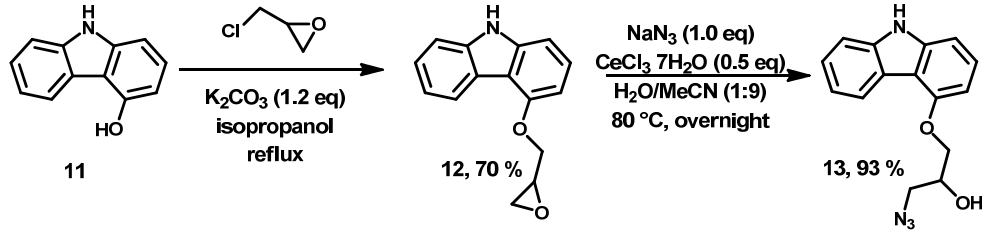
	Time (h)	Conversion	ee A (%)	ee B (%)	E
1	24	20%	24	96	83
2	48[a]	32%	47	96	78

[a]Enzyme added in two portions (half at t=0 h, half at t=24 h).

The scope of the one-pot ring opening 'click' reaction can therefore be extended to include the variety of strained cyclic cyclooctyne derivatives that have been developed recently.[26]

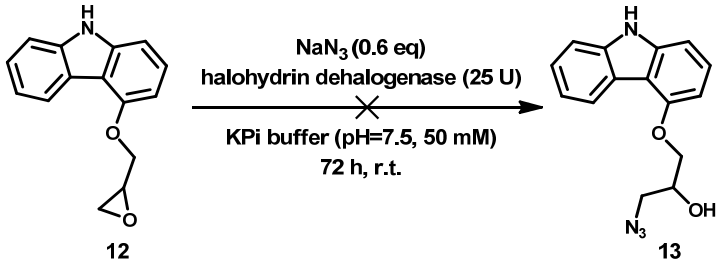
4.5 β-Adrenergic receptor ligands

As aforementioned, the structure of the optically pure β-hydroxytriazoles is interesting in the context of potential β-AR ligands. To test whether these would be converted by halohydrin dehalogenase to produce the corresponding azidoalcohols, the racemic epoxide **12** was synthesized (Scheme 4). Hydroxycarbazole **11** was reacted with 1.2 eq of epichlorohydrin in the presence of potassium carbonate to afford **12**. Epoxide **12** underwent $CeCl_3 \cdot 7H_2O$ promoted azidolysis to give the desired 1,2-azidoalcohol **13**.[27]



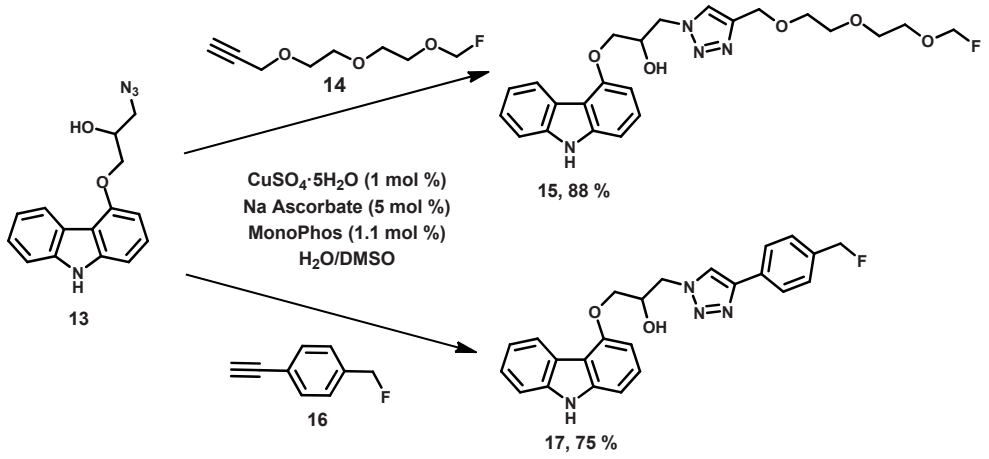
Scheme 4 Synthesis of racemic carbazol epoxide **12** and azidoalcohol **13**

With the racemic reference compounds **12** and **13** in hand, epoxide **12** was subjected to the optimized reaction conditions for the enzymatic azidolysis (Scheme 5). Unfortunately, under these conditions, none of the desired product **13** could be detected, and the starting material could be recovered in nearly quantitative amounts even after 72 h.



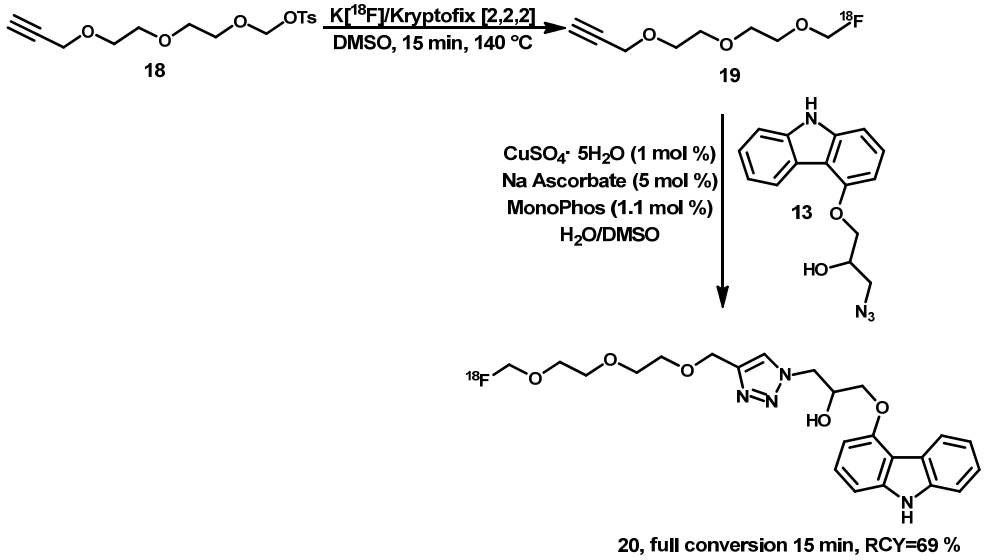
Scheme 5 Attempted enzymatic conversion of **12**

Nevertheless, the structure of azidoalcohol **13** is still one that inspires interest with regards to potential properties as a ligand for β-ARs, thus the synthesis was continued with racemic compounds. Two fluorine containing triazoles **15** and **17** were synthesized as cold reference compounds for corresponding [^{18}F] containing tracers (Scheme 6).



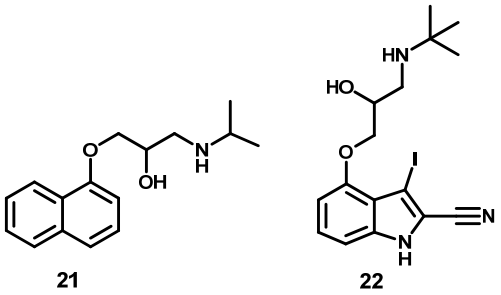
Scheme 6 'Click' reactions to yield fluorine containing triazoles

With cold reference compounds in hand, we proceeded to label azidoalcohol **13** with a [^{18}F]-alkyne. Starting from a tosylated alkyne **18**, PEGylated alkyne **19** could be isotopically labeled by nucleophilic substitution with an [^{18}F] anion (Scheme 7). Subsequent cycloaddition to azidoalcohol **13** gave the desired triazole **20**, ([^{18}F]-PEG-triazole-carazolol or [^{18}F]FPTC) with a radiochemical yield of 69%.



Scheme 7 Synthesis of tracer [^{18}F]FPTC (**20**)

The tracer was tested on a C6 glioma rat cell line to ascertain the binding affinity of [^{18}F]FPTC to β-AR. It is important to note that typically the *(S)*-enantiomer of β-adrenergic receptor ligands tends to have significantly better affinity, and in this case, the racemic compound was being measured. The cells were incubated with the tracer for 1 h at 37°C alongside varying concentrations of the cold blocker propranolol **21** (100 pM-50 μM).[28] The same competition experiment was performed using the gold standard iodocyanopindolol **22** (ICYP) (Scheme 8) labeled with [^{125}I].[29] This allowed for the comparison of the new tracer with a known high affinity ligand for β-ARs under the same conditions (cell line and procedure).



Scheme 8 Propranolol and iodocyanopindolol

The remaining activity, after thorough washing to remove any unbound tracer, was calculated using a gamma-counter and corrected for cell number. This allowed for the IC_{50} value of [^{18}F]FPTC to be determined to be 103 nM. ^{125}ICYP gave an IC_{50} value of 52 nM. From these values we can conclude that [^{18}F]FPTC shows high specific binding to the β-AR receptors. The tracer was incubated at 37°C to test its stability, and negligible degradation was detected after 120 min. The logarithmic value of the partition coefficient (log P) of [^{18}F]FPTC was determined in octanol and phosphate buffered saline (PBS) and determined to be 2.48. This is in the optimal range of lipophilicity for compounds to cross the BBB (see section 4.1). Based on these *in vitro* results, [^{18}F]FPTC was selected for *in vivo* studies.[30]

Typically, agonists and partial agonists of β-AR require the presence of an aliphatic oxypropanolamine moiety, commonly referred to as the "ligand tail" to achieve high receptor affinity.[31] The amine and hydroxyl groups have been shown to form a conserved hydrogen bond network with several polar residues in the front pocket of the receptor. In [^{18}F]FPTC, the hydroxyl group remains, however, there is no secondary amine. It is known that the triazole moiety is an amide bond bioisostere, with the C5 proton behaving as a proton donor, much in the way that the amine of an amide bond does (see Chapter 1, Figure 2).[32] Thus in a sense, the "ligand tail" is present in a new form, with the triazole C5 proton in the position of the amino proton.

4.6 Conclusions

A methodology has been developed which combines the enzymatically catalyzed enantioselective azidolysis of aromatic epoxides to the corresponding azidoalcohols, and the tandem 'click' reaction to azides to yield β-hydroxytriazoles. The mild reaction conditions involve an aqueous medium at neutral pH and room temperature. The one-pot nature of the process allows for a simpler, faster and more environmentally friendly reaction, work-up and purification. The biotransformation retains its high level of

selectivity and the 'click' step of the tandem process can be promoted either through copper catalysis or by a copper-free procedure taking advantage of ring strain, opening the possibility for a wide variety of applications.

Using this 1,2-azidoalcohol motif, a new tracer [^{18}F]FPTC was designed, synthesized, and tested for its affinity for β-ARs. Although the biotransformation to yield a single enantiomer of the product was unsuccessful, the racemate proved simple to synthesize and straightforward to label giving [^{18}F]-containing compounds in good radiochemical yields. [^{18}F]FPTC proved to be very stable, and showed promising *in vitro* characteristics. Interestingly, it has a slightly different motif than the typical agonists and antagonists chosen for targeting these receptors, and *in vivo* testing is being pursued.

4.7 Experimental Section

General

$CuSO_4 \cdot 5H_2O$, sodium ascorbate and sodium azide were purchased from Sigma-Aldrich and used as received. MonoPhos was synthesized as described in the literature. Enantioselectivities were determined by HPLC analysis using a Shimadzu LC-10AD*VP* HPLC equipped with a Shimadzu SPD-M10A*VP* diode array detector.

Safety

Working with azides should always be done carefully. Organic azides, particularly those of low molecular weight, or with high nitrogen content, are potentially explosive. Heat, light and pressure can cause decomposition of the azides. Furthermore, the azide ion is toxic, and sodium azide should always be handled while protected with gloves. Heavy metal azides are particularly unstable, and may explode if heated or shaken.

Enzyme Preparation

Halohydrin dehalogenase was expressed in *E. coli* MC1061 using the pBAD vector.[33] Transformed cells were streaked on Luria Broth (LB) agar plates containing ampicillin and incubated overnight at 37 °C. A preculture was started by inoculating 100 mL LB containing 50 μg/ml ampicillin with one colony from the plate. After overnight incubation at 37°C, the preculture was diluted in 1 L of Terrific Broth (TB) containing 50 μg/mL ampicillin, 0.5 M sorbitol and 0.02% arabinose. This main culture was incubated for two days at 37°C. The cells were centrifuged (10 min, 13 000 g), washed, and resuspended in 5 mL/g pellet of TEG buffer (10 mM Tris-SO_4 pH=7.5, 1 mM EDTA and 10% glycerol). Cells were broken by sonication and the extract was centrifuged (16000 g, 45 min, 4°C). The supernatant was applied on a Q-Sepharose column (50 mL, GE Healthcare) and elution was carried out with a gradient of 0 to 0.45 M $(NH_4)_2SO_4$ in TEG. The collected fractions that displayed enzymatic activity were pooled. $(NH_4)_2SO_4$ was added to a concentration of

1.5 M and the protein was applied on a Phenyl-Sepharose column (60 mL, GE Healthcare). Elution was carried out with a gradient of 1.5 M to 0 M $(NH_4)_2SO_4$ in TEG. Fractions that displayed enzymatic activity were pooled and concentrated over a 10 kDa filter (Millipore). The enzyme was 97% pure judged by SDS-PAGE. Purified enzyme was sterilized using a 0.2 μm filter and stored at -70°C. Enzyme assay was carried out as previously described using 1-(*p*-nitrophenyl)-2-bromoethanol.[34] The concentration was determined to be 25 U/mL and the specific activity 20 U/mg.

Buffer Exchange

The enzyme was transferred from Tris-HCl buffer to potassium phosphate buffer (pH 7.5, 50 mM). The Tris-buffer solution was loaded onto a 10 kDa filter (Millipore) in 500 μL fractions and spun down (14,000 g, 30 min, 4ºC). After the protein was loaded, the filter was flushed twice with potassium phosphate buffer (500 μL each) prior to elution from the filter (1,000 g, 3 min, 4ºC).

Octanol/Water Partition Coefficient Study.

Partition coefficients were determined at pH=7.4. 5 μL containing 500 kBq of the radiolabeled compound in phosphate buffered saline (PBS) was added to a vial containing 1.2 mL 1-octanol and PBS (1:1). After vortexing for 1 min, the vial was centrifuged for 5 min at 10 000 rpm to ensure complete separation of layers. Then, 40 μL of each layer was taken in a pre-weighed vial and measured in the γ-counter. Counts per unit weight of sample were calculated.

General Procedure for Biocatalyzed Azidolysis of Epoxides:

To a 15.0 mL Greiner tube was added 1.0 eq of epoxide (0.032 mmol) and 10.0 mL of 50 mM potassium phosphate buffer (pH 7.5). To this solution was added 0.6 eq of sodium azide followed by 0.5 mL of potassium phosphate buffer containing 25 U (initial activity) of halohydrin dehalogenase. The reaction mixture was shaken overnight and the resulting solution extracted with diethyl ether (3 x 5 mL). The organic layers were combined and dried over $MgSO_4$. Solvent was removed under reduced pressure to give the crude reaction products which were analyzed by HPLC.

General Procedure for One-Pot Enzymatic 'Click' Reactions:

Copper Catalyzed Procedure

To a 15.0 mL Greiner tube was added 5 mol % $CuSO_4 \cdot 5H_2O$ and 25 mol % sodium ascorbate followed by 1.0 mL of 50 mM potassium phosphate buffer. To this solution was added 5.5 mol % MonoPhos and the resulting mixture was shaken for 10 min. Sodium

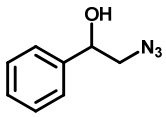
azide (0.6 eq) and epoxide (1.0 eq, 0.032 mmol) were added to the reaction mixture, which was diluted with a further 6 mL of buffer (volume varied depending on the substrate concentration desired). To this solution was added 2.0 eq of alkyne, followed by 0.5 mL of buffer containing 25 U (initial activity) of halohydrin dehalogenase. The Greiner tube was placed on a shaker and after 24 h the reaction mixture was lyophilized overnight. The resulting solid content was flushed over a short plug of silica with ethyl acetate, followed by methanol. Solvent was removed under reduced pressure and the crude reaction mixture was prepared for analysis by HPLC.

Copper Free Procedure

To a 15.0 mL Greiner tube was added 1.0 eq of epoxide (0.032 mmol, 5.0 mg) and 7.0 mL of potassium phosphate buffer (50 mM). To this reaction mixture was added 2.0 eq of cyclooctyne followed by 0.5 mL of potassium phosphate buffer containing 25 U (initial activity) of halohydrin dehalogenase. The reaction vessel was sealed with parafilm and shaken for the indicated amount of time (24 or 48 h). The reaction mixture was then lyophilized overnight and the resulting solid was flushed over silica with ethyl acetate followed by methanol. Solvent was removed under reduced pressure to yield the crude reaction mixture which could be analyzed by HPLC.

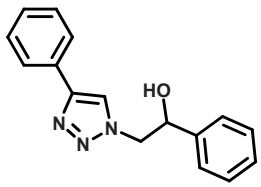
Characterization of substrates and reference compounds

2-Azido-1-phenylethanol (3)



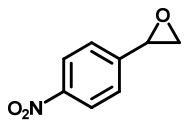
2-Azido-1-phenylethanol was prepared as described in the literature and NMR data was in accordance with the literature.[35] Enantiomeric excess determined by HPLC (Chiralpak OD 99:1 Heptane: *i*-PrOH: t_r= 42.2, 47.0 min). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26-7.39 (m, 5H), 4.89 (dd, *J*=4.4, 3.6 Hz, 1H), 3.47 (m, 2H), 2.40 (br s, 1H).

1-Phenyl-2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethanol. To a 10 mL roundbottom flask was added 10.9 mg (0.07 mmol) of 2-azido-1-phenylethanol and 15 μL (0.13 mmol) of phenylacetylene. $CuSO_4 \cdot 5H_2O$ (0.85 mg, 0.0034 mmol) was weighed into a small sample vial along with Na ascorbate (3.37 mg, 0.017 mmol) and dissolved in 1.0 mL distilled H_2O. To this solution was added MonoPhos (1.34 mg) and the solution was stirred at room temperature for 15 min. The solution in the sample vial was added to the roundbottom flask (with stirring) and a further 2.0 mL of water and 1.0 mL of DMSO were added to the mixture. The reaction mixture

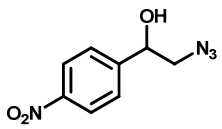


was allowed to stir at room temperature overnight, and in the morning a pale yellow precipitate had formed. 5.0 mL of ice cold distilled water was added to the reaction mixture and the precipitate was filtered off and washed with cold water. The product proved to be sufficiently pure to prevent the need for any further purification steps. Yield=83%. Mp 156-157ºC. Enantiomeric excess determined by HPLC (Chiralcel OD-H 80:20 Heptane: *i*-PrOH: t_r=18.0, 22.2 min). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H), 7.73 (d, *J*=6.8 Hz, 1H), 7.32-7.41 (m, 8H), 5.24 (m, 1H), 4.65 (m1H), 4.46 (m Hz, 1H), 3.61 (s, 1H); ^{13}C NMR (100.59 MHz, d6-DMSO): 146.7, 142.9, 131.7, 129.8, 129.1, 128.6, 128.4, 126.9, 125.9, 122.8, 72.3, 57.6. HRMS (EI) calcd for $C_{16}H_{15}N_3O$ [M+H$^+$] 266.1293, found 266.1288.

2-(4-Nitrophenyl)oxirane (2). 2-Chloro-1-(4-nitrophenyl)ethanol (1.0 mmol, 201.6 mg) was dissolved in 3.0 mL MeOH. 1.0 mL of distilled H_2O was slowly added to the solution with stirring. K_2CO_3 was then added to the solution, and the reaction mixture was allowed to stir at room temperature for a further 3 h. The progress of the reaction was monitored by thin layer chromatography (4:1 pentane:ether). The reaction mixture was poured onto 20 mL of H_2O and extracted with diethyl ether (3 x 10 mL). The organic fractions were collected and dried over $MgSO_4$. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography (pentane:ether 4:1). The resulting product was a yellow solid. Yield=89%. Enantiomeric excess determined by HPLC (Chiralpak AS-H 90:10 Heptane: *i*-PrOH: t_r= 14.8, 18.3 min) or (Chiralcel OD-H 80:20 Heptane: *i*-PrOH: t_r= 12.2, 12.8 min). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (d, *J*=11.2 Hz, 2H), 7.42 (d, *J*=11.2 Hz, 2H), 3.95 (m, 1H), 3.22 (m, 1H), 2.76 (m, 1H); ^{13}C NMR (100.59 MHz, $CDCl_3$): δ 147.7, 145.2, 126.1, 123.6, 51.6, 51.3.

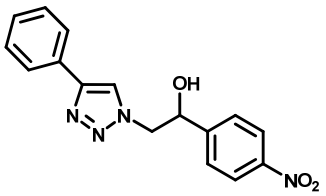


2-Azido-1-(4-nitrophenyl)ethanol (4). 2-Bromo-1-(4-nitrophenyl)ethanol (500.0 mg, 2.25 mmol) was dissolved in 75.0 mL DMF in a 200 mL roundbottom flask. NaN_3 (732.0 mg, 11.3 mmol) was added to solution, and the flask equipped with a condenser was heated to 100ºC. The reaction mixture was left overnight with stirring. The solution was poured onto 50 mL of brine followed by extraction with diethyl ether (3 x 25 mL). The organic layers were collected and dried over $MgSO_4$ and the solvent was removed under reduced pressure. The resulting oil was purified by column chromatography (1:1 pentane:ether) to give the pure azidoalcohol as a yellow oil. Yield=57%. Enantiomeric excess determined by HPLC (Chiralcel OD-H 80:20 Heptane: *i*-PrOH: t_r= 13.0, 13.6 min, or, Chiralpak AS-H 90:10 Heptane: *i*-PrOH: t_r: 28.6, 34.8 min). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, *J*=12.0 Hz, 2H), 7.56 (d, *J*=12.0 Hz, 2H), 4.99 (dd, *J*=4.0, 3.6 Hz, 1H),

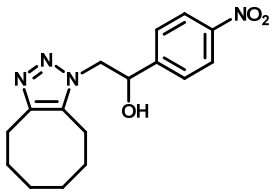


3.49 (m, 2H), 3.00 (br s, 1H); ^{13}C NMR (100.59 MHz, $CDCl_3$): 147.7, 126.8, 123.9, 123.7, 72.3, 57.6.

1-(4-Nitrophenyl)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethanol (6B). To a 10.0 mL roundbottom flask was added 46.9 mg (0.23 mmol) of 2-azido-1-(4-nitrophenyl)ethanol and 46.0 mg (0.05 mL, 0.45 mmol) of phenylacetylene. $CuSO_4 \cdot 5H_2O$ (0.57 mg, 0.0023 mmol) was weighed into a small sample vial along with Na ascorbate (2.28 mg, 0.012 mmol) and dissolved in 1.0 mL distilled H_2O. To this solution was added MonoPhos (0.91 mg, 0.0025 mmol) and the solution was stirred at room temperature for 15 min. The solution in the sample vial was added to the roundbottom flask (with stirring) and a further 2.0 mL of water was added to the mixture. The reaction mixture was allowed to stir at room temperature for 6 h and the progress of the reaction was monitored by thin layer chromotography (2:1 pentane:ether). Upon completion of the reaction, the mixture was poured onto ice water and extracted with DCM (3 x 5 mL). The organic layers were combined, dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield a yellow solid. The solid was washed with chloroform and water and then dried. Yield=74 %. mp 189-190ºC. Enantiomeric excess determined by HPLC (Chiralpak AS-H 80:20 Heptane: *i*-PrOH: t_r= 37.1, 36.7 min). ^{1}H NMR (400 MHz, d6-acetone): δ 8.34 (s, 1H), 8.25 (d, *J*=4.8 Hz, 2H), 7.88 (d, *J*=7.6 Hz, 2H), 7.76 (d, *J*=8.8 Hz, 2H), 7.43 (t, *J*= 7.2 Hz, 2H), 7.34 (t, *J*=7.6 Hz, 1H), 5.43 (m, 2H), 4.78 (dd, *J*=12, 3.6 Hz, 1H), 4.68 (dd, *J*=16, 7.2 Hz, 1H); ^{13}C NMR (100.59 MHz, d6-acetone):149.5, 147.9, 147.0, 131.7, 129.0, 127.9, 127.5, 125.5, 123.6, 121.9, 71.8, 56.9. HRMS (EI) calcd for $C_{16}H_{14}N_4O_3$ [M+H$^+$] 311.1144, found 311.1139. HRMS (EI) calcd for $C_{33}H_{36}O_9N_3S$ [M+H$^+$] 650.2167, found 650.2177.

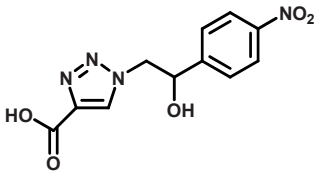


2-(4,5,6,7,8,9-Hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)-1-(4-nitro phenyl) ethanol (10B). 2-Azido-1-(4-nitrophenyl)ethanol (10.7 mg, 0.05 mmol) was dissolved in 1.0 mL dry THF in a Schlenk vessel under N_2 atmosphere. 2.0 eq of cyclooctyne was added and the solution was stirred at room temperature overnight. Progress of the reaction was followed by GC/MS. THF and any remaining cyclooctyne were removed by rotary evaporation and the resulting viscous yellow oil was purified by column chromatography (5:1 pentane: ethyl acetate) to give the product as a yellow oil. Yield= 43%. Enantiomeric excess determined by HPLC (Chiralcel OD-H 80:20 Heptane: *i*-PrOH: t_r= 15.9, 17.2 min). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J*= 8.4Hz, 2H), 7.58 (d, *J*=8.4 Hz, 2H), 5.45 (dd, *J*= 8.0, 3.2 Hz, 1H), 4.43 (dd, *J*=14, 3.2 Hz, 1H), 4.29 (*J*= 13.8, 8.0 Hz, 1H), 2.85-2.89 (br m, 2H), 2.64-2.67 (br m, 2H), 1.64-1.72 (m, 4H), 1.32-1.45 (m, 4H); ^{13}C NMR (100.59 MHz,



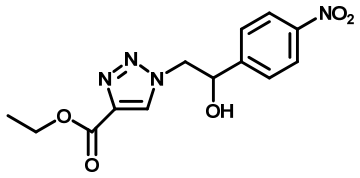
d6-$CDCl_3$): 148.0, 147.6, 127.1, 124.1, 118.7, 113.2, 71.8, 55.4, 27.9, 26.1, 25.9, 24.6, 23.9, 22.2. HRMS (EI) calcd for $C_{16}H_{20}N_4O_3$ [M+H^+]
317.1608, found 317.1608.

1-(2-Hydroxy-2-(4-nitrophenyl)ethyl)-1*H*-1,2,3-triazole-4-carboxylic acid. 2-Azido-1-(4-nitrophenyl)ethanol (50.0 mg, 0.24 mmol) was added to a 10.0 mL roundbottom flask followed by propiolic acid (18.5 mg, 0.26 mmol). In a separate vial, $CuSO_4 \cdot 5H_2O$ (3.0 mg, 0.012 mmol) was dissolved in 1.0 mL distilled H_2O along with Na ascorbate (11.9 mg, 0.06 mmol). MonoPhos (4.7 mg) was then added to the sample vial along with 0.3 mL DMSO. The solution was stirred at room temperature for 15 min. The contents of the sample vial were then added to the round-bottomed flask with stirring and a further 3.0 mL of distilled water and 0.5 mL of DMSO were added. Stirring of the reaction mixture was continued at room temperature overnight. The solution was poured onto ice water and extracted with ethyl acetate (3 x 10 mL). The organic layers were collected and dried over $MgSO_4$. The solvent was removed under reduced pressure and the resulting yellow oil was purified by column chromatography (1:1 pentane: ether). The product could also be purified by recrystallization from methanol to give a white solid. Yield=75%. mp 163-164ºC. Enantiomeric excess determined by HPLC (Chiralpak OD-H 80:20 Heptane: *i*-PrOH: t_r= 23.9, 28.2 min). ^{1}H NMR (400 MHz, CD_3OD): δ 8.48 (s, 1H), 8.21 (d, *J*=8.8 Hz, 2H), 7.64 (d, *J*=8.8 Hz, 2H), 5.26 (dd, *J*=4.0, 3.6 Hz, 1H), 4.76 (dd, *J*=13.8, 3.6 Hz, 1H), 4.63 (dd, *J*=13.8, 8.0 Hz, 1H); ^{13}C NMR (100.59 MHz, CD_3OD):162.7,148.7, 147.8, 129.2, 127.9, 127.1, 123.4, 71.1, 56.9.



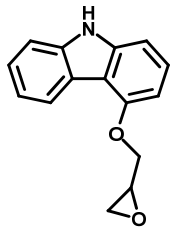
Ethyl-1-(2-hydroxy-2-(4-nitrophenyl)ethyl)-1*H*-1,2,3-triazole-4-carboxylate

2-Azido-1-(4-nitrophenyl)ethanol (50.0 mg, 0.24 mmol) was added to a 10.0 mL roundbottom flask. In a sample vial, $CuSO_4 \cdot 5H_2O$ (3.0 mg, 0.012 mmol) and Na ascorbate (11.9 mg, 0.06 mmol) were dissolved in 1.0 mL of distilled water. To this solution was added 4.7 mg of MonoPhos in 0.3 mL of DMSO. The mixture was stirred at room temperature for 15 min. The solution of the copper complex was then added to the round-bottomed flask and a further 3.0 mL of water and 0.5 mL of DMSO were added. To this stirred solution was added dropwise ethyl propiolate (47.0 mg, 48.5 μL). The yellow solution was allowed to stir at room temperature overnight and in the morning a yellow precipitate had formed. 5.0 mL of ice cold distilled water was added to the solution and the precipitate was filtered off, washed with water and dried. Yield=73 %. mp=152-153ºC. Enantiomeric excess determined by HPLC (Chiralcel OD-H 80:20



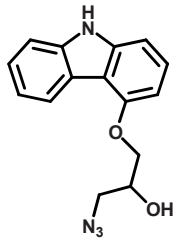
Heptane: *i*-PrOH: t_r= 18.9, 25.2 min). ^{1}H NMR (400 MHz, d6-acetone): δ 8.52 (s, 1H), 8.24 (d, *J*=8.4 Hz, 2H), 7.76 (d, *J*=8.4 Hz, 2H), 5.48 (s, 1H), 5.44 (m, 1H), 4.85 (dd, *J*=14.4, 3.6 Hz, 1H), 4.71 (dd, *J*=16.0, 6.0 Hz, 1H), 4.34 (q, *J*=7.2 Hz, 2H), 1.34 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (100.59 MHz, d6-acetone): 160.2, 149.1, 129.6, 128.4, 127.5, 123.6, 116.1, 71.4, 60.6, 56.8, 13.9. HRMS (EI) calcd for $C_{13}H_{14}N_4O_5$ [M+H$^+$] 307.1042, found 307.1037.

4-(Oxiran-2-ylmethoxy)-9H-carbazole (12)



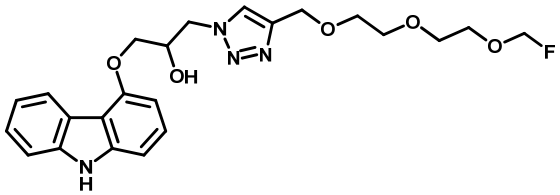
9*H*-Carbazol-4-ol (100.0 mg, 0.55 mmol) was dissolved in 20 mL of isopropanol. To this solution was added epichlorohydrin (75.9 mg, 0.82 mmol) and potassium carbonate (90.5 mg, 0.65 mmol). The reaction mixture was warmed to 80°C and reacted overnight. After cooling the solution, 15 mL of aqueous NH_4Cl was added to the solution and the resulting mixture was extracted with dichloromethane (3 x 15 mL). The organic layers were combined and washed with brine, dried over $MgSO_4$ and the solvent was removed by rotovap. The product was isolated by column chromatography (3:1 pentane:ethyl acetate). R_f=0.75 (1:1 pentane:ethyl acetate). Yield=70%. Yellow solid. The spectroscopic data is in accordance with the literature.[36] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (d, *J*=8.0 Hz, 1H), 8.04 (s, 1H), 7.25-7.42 (m, 4H), 7.01 (d, *J*=8.0 Hz, 1H), 6.64 (d, *J*=12 Hz, 1H), 4.44 (d, *J*=16 Hz, 1H), 4.22 (m, 1H), 3.54 (m, 1H), 2.98 (m, 1H), 2.88 (m, 1H); ^{13}C NMR (100.59 MHz, $CDCl_3$): 155.1, 141.2, 139.0, 126.8, 125.3, 123.4, 122.6, 119.9, 112.9, 110.3, 104.3, 101.5, 69.0, 50.6, 45.1.

1-((9*H*-Carbazol-4-yl)oxy)-3-azidopropan-2-ol (13)



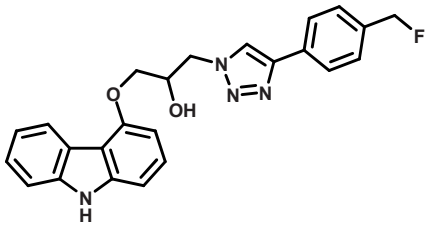
To a mixture of 4-(oxiran-2-ylmethoxy)-9*H*-carbazole (40.5 mg, 0.17 mmol) and $CeCl_3{\cdot}7H_2O$ (22.2 mg, 0.09 mmol) in 4.0 mL of a 9:1 mixture of acetonitrile/water was added NaN_3 (12.1 mg, 0.19 mmol). The resulting solution was stirred at reflux overnight. The reaction mixture was dilutedwith dichloromethane and washed with water (5 mL) and brine (2 x 5 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product was purified by column chromatography (2:1 pentane:ethyl acetate). R_f=0.8. Yield=93%. The spectroscopic data is in accordance with the literature.[37] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (d, *J*=8.0 Hz, 1H), 8.07 (s, 1H), 7.26-7.44 (m, 4H), 7.02 (d, *J*=8.0 Hz, 1H), 6.61 (d, *J*=8.0 Hz, 1H), 4.33 (m, 1H), 4.20 (d, *J*=8.0 Hz, 2H), 3.60 (m, 2H), 2.78 (br s, 1H); ^{13}C NMR (100.59 MHz, $CDCl_3$): 154.4, 140.8, 138.6, 126.4, 124.9, 122.5, 121.9, 119.4, 112.2, 110.1, 104.1, 100.8, 69.4, 69.0, 53.6.

1-((9*H*-Carbazol-4-yl)oxy)-3-(4-((2-(2-(fluoromethoxy)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)propan-2-ol (15)



1-((9*H*-carbazol-4-yl)oxy)-3-azidopropan-2-ol (6.0 mg, 0.021 mmol), and 3-(2-(2-(fluoromethoxy)ethoxy)ethoxy)prop-1-yne (7.8 mg, 0.043 mmol) were dissolved in 3.0 mL of a 4:1 mixture of water:DMSO. To this solution was added 5 mol % $CuSO_4 \cdot 5H_2O$ (1.0×10^{-3} mmol), 25 mol % sodium ascorbate (5.25×10^{-3}mmol) and 5.5 mol % MonoPhos (1.12×10^{-3} mmol). The reaction mixture was allowed to stir at room temperature and the progress of the reaction was followed by thin layer chromatography. Upon complete consumption of the azidoalcohol, the reaction mixture was diluted with water and extracted with DCM. The organic layer was dried over $MgSO_4$ and the product was purified by column chromatography (2:1 pentane:ethyl acetate) to give a white solid. R_f=0.15 (1:1 pentane:ethyl acetate).Yield=88%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J*=8.0 Hz, 1H), 8.20 (s, 1H), 7.70 (s, 1H), 7.41-7.45 (m, 2H), 7.24-7.27 (m, 2H), 7.09 (d, *J*=8.0 Hz, 1H), 6.62 (d, *J*=8.0 Hz, 1H), 4.77-4.80 (m, 1H), 4.64-4.67 (m, 4H), 4.58 (t, *J*=4.0 Hz, 1H), 4.46 (t, *J*=4.0 Hz, 1H), 4.22 (d, *J*=4.0 Hz, 2H), 3.73 (t, *J*=8.0 Hz, 1H), 3.63-3.67 (m, 8H), 3.30 (br s, 1H). ^{13}C NMR (100.59 MHz, $CDCl_3$): 154.3, 141.0, 138.7, 126.7, 125.2, 122.6, 122.1, 119.8, 112.5, 110.4, 104.4, 101.2, 70.7, 70.5, 70.4, 69.6, 69.1, 68.8, 64.5, 53.4; ^{19}F NMR (200 MHz, $CDCl_3$): 42.6 (m).

1-((9*H*-Carbazol-4-yl)oxy)-3-(4-(4-(fluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)propan-2-ol (17)



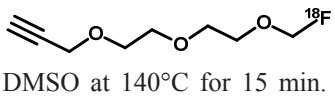
1-Ethynyl-4-(fluoromethyl)benzene (37.0 mg, 0.30 mmol) and 1-((9*H*-carbazol-4-yl)oxy)-3-azidopropan-2-ol (42.3 mg, 0.15 mmol) were dissolved in a mixture of H_2O/DMSO (4:1). To this mixture was added 1 mol % of $CuSO_4 \cdot 5H_2O$ (0.37 mg), 5 mol % of sodium ascorbate (1.49 mg) and 1.1 mol % of MonoPhos (0.59 mg). The reaction mixture was stirred until the disappearance of azidoalcohol was visible by thin layer chromatography. The reaction mixture was extracted with DCM and the organic layer was dried over $MgSO_4$. The product was purified by column chromatography (2:1 pentane:ethyl acetate) to yield a white solid. R_f=0.2 (1:1 pentane:ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (s, 1H), 8.22 (d, *J*=8.0 Hz, 1H), 7.87 (s, 1H), 7.74 (d, *J*=8.0 Hz, 2H), 7.37-7.43 (m, 4H), 7.23-7.31 (m, 2H), 7.07 (d, *J*=8.0 Hz, 1H), 6.60 (d, *J*=4.0 Hz, 1H), 5.43 (s, 1H), 5.31 (s, 1H), 4.83 (m, 1H),

4.62-4.67 (m, 2H), 4.23 (d, *J*=4.0 Hz, 2H), 3.72 (br s, 1H); ^{13}C NMR (100.59 MHz, $CDCl_3$): 154.4, 147.2, 141.0, 138.8, 136.1, 135.9, 130.7, 128.0, 126.7, 125.7, 125.3, 122.5, 122.1, 121.6, 112.4, 110.2, 104.4, 101.3, 85.1, 83.5, 69.2, 68.9, 53.1; ^{19}F NMR (200 MHz, $CDCl_3$): 56.6 (t, *J*=52.0 Hz).

Radiochemistry General

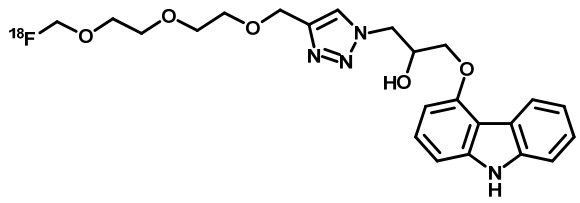
Chemicals and solvents were obtained from commercial sources and were of analytical grade. Pindolol, (*R*,*S*)- and (*S*)-propranolol were purchased from Sigma, St. Louis, USA. A Phenomenex Prodigy C_{18} (250mm x 10 mm, 5 µm) HPLC column was used for HPLC analysis. For metabolite studies, a Radial-Pak C_{18} HPLC column (100mm x 8mm i.d., 10µm, Waters, USA) was used. Aqueous [^{18}F]-fluoride was produced by irradiation of [^{18}O] water with a Scanditronix MC-17 cyclotron via the 18O (p,n)18F nuclear reaction. The [^{18}F]-fluoride solution was passed through a SepPak Light Accell plus QMA anion exchange cartridge (Waters) to recover the ^{18}O-enriched water. [^{18}F]-fluoride was eluted from the cartridge with 1 mL of K_2CO_3 (4.5 mg/mL) and collected in a vial with 20 mg Kryptofix 2.2.2. To this solution, 1 mL of acetonitrile was added and the solvents were evaporated at 130°C. The [^{18}F]KF/ Kryptofix complex was dried 3 times by the addition of 0.5 mL of acetonitrile, followed by evaporation of the solvent.

[^{18}F]-PEGylated alkyne (19)



Compound **19** was prepared by [^{18}F]-fluorination of the corresponding tosylate **18** with K[^{18}F]/kryptofix [2,2,2] in DMSO at 140°C for 15 min. The product was diluted with 20 mL of water and passed through a C18 cartridge (activated with 5 mL EtOH and 10 mL H_2O). The product was eluted from the cartridge with 4 mL of water and purified by HPLC (10% EtOH in NaH_2PO_4 0.025 M (pH 7).

[^{18}F]FPTC (20)



Compound **20** was synthesized by a copper catalyzed alkyne-azide cycloaddition reaction of [^{18}F]-PEGylated alkyne **19** with azidoalcohol **13**. Alkyne **19** and azidoalcohol **13** were dissolved in a 4:1 mixture of water:DMSO. To this solution was added a solution containing $CuSO_4 \cdot 5H_2O$ (1 mol %), sodium ascorbate (5 mol %) and MonoPhos (1.1 mol %) and the resulting solution was stirred at room temperature. Upon completion of the reaction, purification with radio-HPLC was performed (4 mL/min; retention time [^{18}F]FPTC: 16

min). The radiolabeled compound [^{18}F]FPTC was obtained in 35% radiochemical yield from [^{18}F]-fluoride (decay-corrected) in 55 min. At the end of synthesis, the specific activity was 120 GBq/μmol and the radiochemical purity was >96%.

Stability of [^{18}F]-FPTC

Samples of [^{18}F]FPTC were taken for stability testing. The sample of FPTC was dissolved in PBS (1 mL) and incubated at 37°C. After 60 min, the solution was analyzed by HPLC (retention time: 16 min) (20% CH_3CN/NaH_2PO_4 0.025 M (pH 7); 4 mL/min). A sample of radiolabeled [^{18}F]FPTC was dissolved in PBS (1 mL) and rat plasma (1 mL) and incubated at 37°C. After 1 h and 3 h of incubation, respectively, the stability of the radiolabeled [^{18}F]FPTC was followed by Radio-TLC (eluent: $MeOH/CH_2Cl_2$ 2:8 + 0.1% Et_3N). After elution, the TLCs were analyzed by phosphor storage imaging. The screens were scanned with Cyclone phosphor storage system (PerkinElmer), and the percentage of conversion of [^{18}F]FPTC as a function of the incubation period was calculated by ROI analysis using Opti- Quant software. In order to avoid underestimating the amounts of [^{18}F]FPTC due to its volatile nature, we created a calibration curve of the amount of [^{18}F]FPTC evaporated over time in the radio TLC (y) -0.2141x + 103.11, $R2$) 0.72, P) 0.03) and corrected all values. The calibration curve was prepared by pipetting 1.5 μL of a stock [^{18}F]FPTC solution at different time points in a TLC plate. The TLC was read out by storage phosphorus imaging. Images were analyzed by Optiquant software and the percentage of evaporated activity was calculated.

Distribution Coefficient (LogP)

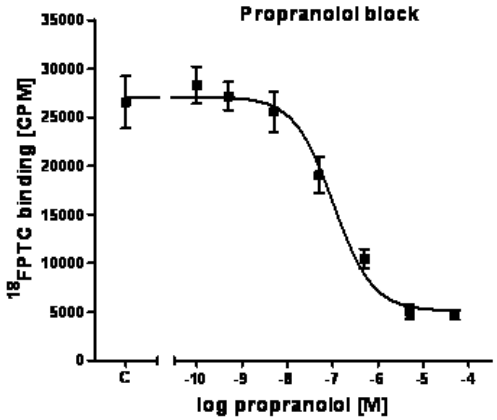
To determine the LogP of [^{18}F]FPTC, an aliquot of 1 mL of HPLC purified [^{18}F]FPTC solution was added to a mixture of *n*-octanol/PBS (5 mL/5 mL) at pH 3 and 7. The tubes were vortexed at room temperature for 1 min, followed by 30 min shaking in a water bath at 37°C. Aliquots of 1000 and 25 μL were drawn from the *n*-octanol and aqueous phase, respectively, and the radioactivity was counted using an automated gamma counter. The experiments were performed in triplicate.

Cellular Uptake of [^{18}F]-FPTC in C6 Glioma Cells

The binding properties of [^{18}F]FPTC were tested *in vitro* in β-AR-expressing C6-glioma cells and compared to the standard β1-/ β2-AR ligand, ^{125}I-iodocyanopindolol and propanolol. C6 glioma cells were maintained in 5 mL Dulbecco's Modified Eagle Medium (DMEM) supplemented with 7.5% fetal bovine serum (FBS) in 25 mL culture flasks. Cells were grown in a humidified atmosphere containing 5% CO2 and were passaged every 3-4

days. For uptake experiments, cells were plated in triplicate in a 12-well plate at a density of 7×10^5 cells per well.

Binding studies (performed 48 h after cell plating, confluence 80-90%) C6 glioma cells were incubated with: 0.41 Bq/ml (5 pM), 4.1 Bq/ml (50 pM) and 8.2 Bq/ml (100 pM) [^{125}I]-ICYP in presence of various concentrations of unlabeled competitors (propranolol and FPTC) (~ 1h). Cell monolayers were washed (3 times) with cold PBS (to remove unbound tracer), detached from the culture plates and transferred to a new tubes. The uptake was assessed using a γ-counter (Compugamma 1282 CS; LKB-Wallac).



Scheme 9 Testing the binding affinity of 18FPTC on glioma cells. IC50= 103 nM

4.8 References and Notes

(1) A. Dömling, I. Ugi. *Angew. Chem. Int. Ed.* **2000**, *39*, 3168-3210.

(2) D. B. Ramachary, C. F. Barbas III. *Chem. Eur. J.* **2004**, *10*, 5323-5331; K. Odlo, E. A. Høydahl, T. V. Hansen. *Tetrahedron Lett.* **2007**, *48*, 2097-2099; K. Barral, A. D. Moorhouse, J. E. Moses. *Org. Lett.* **2007**, *9*, 1809-1811; A. Prasa, W. Dehaen, V. V. Fokin, E. V. der Eycken. *Org. Lett.* **2004**, *6*, 4223-4225.

(3) G. Kumaraswamy, K. Andamma, A. Pitchaiah. *J. Org. Chem.* **2007**, *72*, 9822-9825.

(4) H. Ankati, Y. Yang, D. Zhu, E. R. Biehl, L. Hua. *J. Org. Chem.* **2008**, *73*, 6433-6436.

(5) N. Kasai, T. Suzuki, Y. Furukawa. *J. Mol. Cat:B* **1998**, *4*, 237-252; H. M. S. Assis, A. T. Bull, D. J. Hardman. *Enzyme Microb. Technol.* **1998**, *22*, 545-551; J. H. Lutje

Spelberg, J. E. T. van Hylckama Vlieg, T. Bosma, R. M. Kellogg, D. B. Janssen. *Tetrahedron: Asymmetry* **1999**, *10*, 2863-2870.

(6) R. J. Fox, S. C. Davis, E. C. Mundorff, L. M. Newman, V. Gavrilovic, S. K. Ma, L. M. Chung, C. Ching, S. Tam, S. Muley, J. Grate, J.Gruber, J. C. Whitman, R. A. Sheldon, G. W. Huisman. *Nat. Biotechnol.* **2007**, *25*, 338-344; G. Hasnaoui-Dijoux, E. M. Majeric, J. H. Lutje Spelberg, B. Hauer, D. B. Janssen. *ChemBioChem* **2008**, *9*, 1048-1051.

(7) J. H. Lutje Spelberg, J. E. T. van Hylckama Vlieg, L. Tang, D. B. Janssen, R. M. Kellogg. *Org. Lett.* **2001**, *3*, 41-43.

(8) J. E. T. van Hylckama Vlieg, L. Tang, J. H. Lutje Spelberg, T. Smilda, G. J. Poelarends, T. Bosma, A. E. J. van Merode, M. W. Fraaije, D. B. Janssen. *J. Bacteriol.* **2001**, *183*, 5058-5066.

(9) L.Tang, J. E. van Hylckama Vlieg, J. H. Lutje Spelberg, M. W. Fraaije, D. B. Janssen. *Enzyme and Microbiol. Tech.* **2002**, *30*, 251-258.

(10) M. Aufort, J. Hersovici, P. Bouhours, N. Moureau, C. Girard. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1195-1198; Y. Zhou, Y. Zhao, K. M. O'Boyle, P. V. Murphy. *Bioorg. Med. Chem. Lett.* **2008,** *18*, 954-958; A. Brik, J. Alexandratos, Y.- C. Lin, J. H. Elder, A. J. Olson, A. Wlodawer, D. S. Goodsell, C. -H. Wong. *ChemBioChem* **2005**, *6*, 1167-1169.

(11) S. Su, J. R. Giguere, S. E. Schaus, J. A. Porco. *Tetrahedron* **2004**, *60*, 8645-8657.

(12) A. Pazos, A. Probst, J. M. Palacios, *Brain. Res.* **1985**, *358*, 324-328.

(13) F. de Paermentier, S. C. Cheetham, M. R. Crompton, C. L. Katona, R. W. Horton. *Brain Res.* **1990**, *525*, 71-77.

(14) R. N. Kalaria, A. C. Andorn, M. Tabaton, P. J. Whitehouse, S. I. Harik, J. R. Unnerstall. *J. Neurochem.* **1989**, *53*, 1772-1781.

(15) A. van Waarde, W. Vaalburg, P. Doze, F. J. Bosker, P. H. Elsinga. *Curr. Pharm. Des.* **2004**, *10*, 1519-1536.

(16) P. Doze, P. H. Elsinga, B. Maas, A. van Waarde, T. Wegman, W. Vaalburg. *Neurochem. Int.* **2002**, *41*, 17-27; L. Zheng, M. S. Berridge, P. Ernsberger. *J. Med. Chem.* **1994**, *37*, 3219-3230; A. van Waarde, T. J. Visser, P. H. Elsinga, . M. de Jong, T. W. van der Mark, J. Kraan, K. Ensing, J. Pruim, A. T. M. Willemsen, O. E. Brodde, G. M. Visser, A. M. J. Paans, W. Vaalbur. *J. Nucl. Med.* **1997**, *38*, 934-939.

(17) P. Doze, P. H. Elsinga, E. F. de Vries, A. van Waarde, W. Vaalburg. *Nucl. Med. Biol.* **2000**, *27*, 315-319.

(18) L. S. Campbell-Verduyn, L. Mirfeizi, R. A. Dierckx, P. H. Elsinga, B. L. Feringa. *Chem. Commun.* **2009**, 2139-2141.

(19) M. K. Moi, C. F. Meares, M. J. McCall, W. C. Cole, S. J. DeNardo. *Anal. Biochem.* **1985**, *148*, 249-253.

(20) Under these conditions, after 72 h, no evidence of copper catalyzed azide ring opening of the epoxides could be detected.
(21) Unpublished data. The CuAAC performed with identical conditions, but once in phosphate buffer, and once in distilled water, demonstrated that distilled water as a medium resulted in faster conversion to the triazole product.
(22) The possibility of enzyme inhibition by the azidoalcohol product at higher concentrations cannot be excluded.
(23) L. Brandsma, H. D. Verkruijsse. *Synthesis* **1978**, 290; A. Waldemar, S. G. Bosio, B. Fröhling, D. Leusser, D. Stalke. *J. Am. Chem. Soc.* **2002**, *124*, 8316-8320.
(24) J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli, C. R. Bertozzi. *Proc. Natl. Acad. Sci.* **2007**, *104*, 16793-16797.
(25) A. J. J. Straathof, J. A. Jongejan. *Enzyme Microb. Technol*. **1997**, *21*, 559-571.
(26) J. F. Lutz. *Angew. Chem. Int. Ed.* **2008**, *47*, 2182-2184; J. A. Codelli, J. M. Baskin, N. J. Agard, C. R. Bertozzi. *J. Am. Chem. Soc.* **2008**, 130*,* 11486-11493; X. Ning, J. Guo, M. A. Wolfert and J. G. Boons. *Angew. Chem. Int. Ed.* **2008**, *47*, 2253-2255; S. Sawoo, P. Dutta, A. Chakraborty, R. Mukhopadhyay, O. Bouloussa, A. Sarkar. *Chem. Commun.* **2008**, 5957-5959; S. S. van Berkel, A. J. Dirkes, M. F. Debets, F. L. van Delft, J. J. L. Cornelissen, R. J. M. Nolte, F. P. J. Rutjes, *ChemBioChem* **2007**, *8*, 1504-1508; L. S. Campbell-Verduyn, P. H. Elsinga, L. Mirfeizi, R. A. Dierckx, B. L. Feringa. *Org. Biomol. Chem.* **2008**, *6*, 3461-3463; S. S. van Berkel, A. J. Dirks, S. A. Meeuwissen, D. L. L. Pingen, O. C. Boerman, P. Laverman, F. L. van Delft, J. J. L. Cornelissen, F. P. J. Rutjes. *ChemBioChem*, **2008**, *9*, 1805-1815; C. R. Becer, R. Hoogenboom, U. S. Schubert. *Angew. Chem. Int. Ed.* **2009**, *48*, 2-27.
(27) G. Sabitha, R. S. Babu, M. Rajkumar, J. S. Yadav. *Org. Lett.* **2002**, *4*, 343-345.
(28) G. Sager, B. E. Bang, M. Pedersen, J. Aarbakke. *J. Leukoc. Biol.* **1988**, *44*, 41-45.
(29) O. -E. Brodde, K. Karad, H. -R. Zerkowski, N. Rohm, J. C. Reidemeister. *Circ. Res.* **1983**, *53*, 752-758.
(30) Work performed by Leila Mirfeizi, University Medical Center, University of Groningen.
(31) D. Wacker, G. Fenalti, M. A. Brown, V. Katritch, R. Abagyan, V. Cherezov, R. C. Stevens. *J. Am. Chem. Soc.* **2010**, *132*, 11443-11445.
(32) V. D. Bock, H. Hiemstra, J. H. van Maarseveen. *Eur. J. Org. Chem.* **2006**, 51-68.
(33) R. M. Haak, F. Berthiol, T. Jerphagnon, A. J. Gayet, C. Tarabiono, C. P. Postema, V. Ritleng, M. Pfeffer, D. B. Janssen, A. J. Minnaard, B. L. Feringa, J. G. de Vries. *J. Am. Chem. Soc.* **2008**, *130*, 13508-13509; L. Tang, J. E. van Hylckama Vlieg, J. H. Lutje Spelberg, M. W. Fraaije and D. B. Janssen. *Enzyme and Microbiol. Tech.* **2002**, *30*, 251-258.
(34) J. H. Lutje Spelberg, L. Tang. M. van Gelder, R. M. Kellogg, D. B. Janssen. *Tetrahedron: Asymmetry* **2002**, *13*, 1083-1089.

(35) M. Ueno, C. Hori, K. Suzawa, M. Ebisawa, Y. Kondo. *Eur. J. Org. Chem.* **2005**, 1765-1768.
(36) S. L. Heald, P. W. Jeffs, T. N. Lavin, P. Nambi, R. J. Lefkowitz, M. G. Caron. *J. Med. Chem.* **1983**, *26*, 832-838.
(37) G. Madhusudhan, B. A. Kumar, U. S. Chintamani, M. N. Rao, D. Udaykiran, T. Suresh, V. K. Kumar, K. Mukkanti. *Indian J. Chem. B Org.* **2010**, *49*, 606-610.